REMARKS

Applicants here respond to the Examiner's rejections from the Office Action dated August 1, 2007 (the "Action"). As a preliminary matter, Applicants thank the Examiner for their ongoing work on this case. Further, Applicants thank the Examiner for noting the acceptability of the changes made by Applicants in the previous response directed to trademark usage, and 112, 2nd paragraph. The sole remaining issue is thus the non-obviousness of Applicants' presently claimed invention.

Here, Applicants further clarify the non-obvious characteristics of the presently claimed invention, in view of the references the Examiner has cited against Applicants. Additionally, Applicants provide and discuss a reference illustrating the skill of the art in the area of multiplexed PCR, to further emphasize the non-obviousness of the presently claimed invention.

For the purposes of expediting prosecution, and without acquiescing to the Examiner's rejections, Applicants here have canceled claims 4, 19, 20, 21, 24-30, and 32. The remaining claims under examination are 1-3, 5, 6, 8, 9, 22, 23, 43, and 44. Claim 1 is the only independent claim under examination.

Reconsideration and allowance is respectfully requested in view of the currently presented claims in light of the arguments below.

Rejection of Claims under 35 U.S.C. 103

The Examiner has rejected Claims 1-2, 4-6, 8-9, 43 and 44 as allegedly being obvious over Heid et al., Ohnishi et al, and First et al. In addition, the Examiner has separately rejected Claims 1-4, 19-28, and 32, as being obvious over Dolganov in view of First et al. Since only claims 1-3, 5, 6, 8, 9, 22, 23, 43, and 44 remain under consideration, only these claims will be addressed below.

As the Examiner is aware, in order to establish a prima facie case of obviousness, each of the elements recited in the claims must be taught in the prior art, there should be some reason for one of skill in the art to combine the elements as recited in the claims, and one of skill in the art must have a reasonable expectation of success that the proposed combination would work. Applicants respectfully assert that none of these three requirements are met. As such, a prima facie case of obviousness has not been established.

All Elements of the Presently Claimed Invention are Not Taught in the Cited References

Claim 1 of Applicants' presently claimed invention is Applicants' sole independent claim remaining under examination. Claim 1 provides for a multiplexed PCR amplification reaction, comprising the following element:

> said ninety-five to one-thousand and thirteen oligonucleotide probes labeled with a labeling system suitable for monitoring the amplification reaction as a function of time, each of which is complementary to a region of a different amplified target gene sequence of interest. . .

In addressing this element of Applicants' claimed invention in the Action, the Examiner appears to discuss a previous version of the Applicants claim, and cites to page 987 par. 3 of Heid. Action at p. 4. However, consultation of this section of Heid, in view of Applicants' presently claimed invention, reveals that Heid does not teach "ninety-five to one-thousand and thirteen oligonucleotide probes labeled with a labeling system suitable for monitoring the amplification reaction as a function of time, each of which is complementary to a region of a different amplified target gene sequence of interest." Rather, Heid teaches a collection of single-plex amplification reactions, each occurring in a separate well of a microplate. Each reaction does appear to have a single labeling system which comprises an oligonucleotide probe complementary to a region of a target. However, Heid does not teach a highly multiplexed PCR comprising ninety-five to one thousand and thirteen oligonucleotide probes and ninety-five to one thousand and thirteen primer pairs, as does Applicants presently claimed invention. For reasons to be more fully discussed below, Heid would have no motivation or reason to do so, nor would Heid have had a reasonable expectation that such an approach would work.

The Examiner also rejects claim 1 in view of Doganov. To reiterate, Claim 1 provides for a multiplexed PCR amplification reaction, comprising the following element:

> said ninety-five to one-thousand and thirteen oligonucleotide probes labeled with a labeling system suitable for monitoring the amplification reaction as a function of time, each of which is complementary to a region of a different amplified target gene sequence of interest.

In addressing this element of Applicants' claimed invention in the Action, the Examiner cites to par. 2, as well as p. 1474, par. 1, and Fig 1. Action at p. 10. The Examiner states regarding par. 2 that "where real time amplification PCR using 200 gene-specific primers is taught this inherently involves use of a lab[]led probe labeled with a labeling system suitable for monitoring the amplification reaction as a function of time". However, consultation of these sections of Doganov, in view of Applicants' presently claimed invention, reveals that Doganov does not teach "ninety-five to one-thousand and thirteen oligonucleotide probes labeled with a labeling system suitable for monitoring the amplification reaction as a function of time, each of which is complementary to a region of a different amplified target gene sequence of interest." This is perhaps most easily illustrated by reference to Figure 1 of Doganov. Here, the second box down is shown saying "Multiple Gene Amplicons". Note the presence of the double stranded amplicons, and widgets indicating primers. However, no-where in this figure are there "95-1023 oligonucleotide probes. . .each...complementary to a region a different amplified target gene sequence of interest". Rather, Doganov teaches a collection of single-plex amplification reactions, each occurring in a separate well of a microplate (see bottom portion of Figure 1). Each reaction does appear to have a *single* labeling system which comprises an oligonucleotide probe complementary to a region of a target. However, Applicants claim 95-1023 oligonucleotide probes complementary with their regions of interest, which is simply not present in Doganov.

The Examiner also cites Ohnishi et al, and First et al. in the obviousness rejection. However, neither of these references, alone or in combination, with Heid or Doganov, teach a multiplexed PCR in which "said ninety-five to one-thousand and thirteen oligonucleotide probes labeled with a labeling system suitable for monitoring the amplification reaction as a function of time, each of which is complementary to a region of a different amplified target gene sequence of interest..." Since all of Applicants' claim elements cannot be found in any of the cited references, alone or in combination, the obviousness rejection cannot stand.

Applicants note that certain of the Examiner's arguments regarding certain dependant claims are not addressed since they are believed moot in light of Applicants' independent claim. By not addressing these arguments, Applicants in no way acquiesce to them.

<u>The Combination of References Proposed by the Examiner would not Provide a Reasonable Expectation of Success</u>

The field of molecular biology, generally speaking, is considered an uncertain art. In the context of multiplexed PCR, it is generally recognized that increasing the number of target

nucleic acids to be analyzed results in a concomitant increase in the number of undesired side reactions. For example, primer dimer formation can rapidly swamp out the desired amplification reactions, thus polluting a PCR with unwanted side products. To further support the above declaration, Applicants submit herewith a publication by Rudi et al. (hereinafter "Rudi") that corroborates the above characterizations of the knowledge of one of skill in the art. (Rudi et al., Nucleic Acids Research, 2003, Vol. 31, No. 11 e62). For example, the Introduction of Rudi begins:

> DNA amplification techniques, in particular the polymerase chain reaction (PCR) (1), have become key diagnostic tools. Challenges with PCR, however, are still to obtain quantitative information, and to analyse several targets simultaneously. Developments of multiplex PCR are generally limited by the complexity of the amplification reaction. The number of possible primer pair combinations increases arithmetically with the number of primers present in the reaction, and leads to distorting side reactions. These background amplifications together with differences in amplification efficiencies between amplicons represent severe challenges with multiplex PCR (2).

Rudi et al., then go on to describe a new multiplexed method, involving the laborious nuclease-mediated removal of unincorporated primers as a way of minimizing unwanted side reactions. The authors employ their approaches to achieve multiplex reactions of eight-plex, and twelve-plex. The Examiner is invited to consider Rudi as one illustrative teaching of the difficulties of performing accurate multiplexed PCR, at a time period *AFTER* Applicants' claimed invention.

Thus, Applicants assert that performing a multiplexed PCR with 95-1013 different primer pairs, along with "ninety-five to one-thousand and thirteen oligonucleotide probes labeled with a labeling system suitable for monitoring the amplification reaction as a function of time, each of which is complementary to a region of a different amplified target gene sequence of interest", would not have been expected reasonably likely to succeed at the time of Applicants' invention.

Applicants were able to achieve not only a highly multiplexed PCR with a large number of primers, but *the multiplexed PCR also contains oligonucleotide probes* (e.g. TaqMan probes). Applicants respectfully submit that one of skill in the art at the time of Applicants' invention would have reasonably concluded that adding oligonucleotide probes to

an already highly multiplexed PCR would merely produce a lot of unwanted side products, and simply would not work. The Rudi submission supports this contention.

There is No Motivation to Combine the References Proposed by the Examiner to Produce Applicants Claimed Invention

Finally, there is no motivation to add oligonucleotide probes to a multiplexed PCR. As Heid indicates, oligonucleotide probes (e.g. TaqMan probes) allow for the specific detection of a particular target sequence. The label probes in Heid and Doganov produce a signal when the target is amplified, and the oligonucleotide probe is cleaved as a result of its specific hybridization with the target sequence.

Applicants claim a highly multiplexed PCR containing a large number (95-1023) of not only primer pairs, but also 95-1023 oligonucleotide probes. One of skill in the art would have no motivation for doing such a thing, since no way of discriminating 95-1023 oligonucleotide probes would be achievable in the multiplex PCR. That is, 95-1023 different labels don't readily exist that are capable of selective detection by current instrumentation. Thus, there would be no motivation to include them in the reaction. It would be economically wasteful to do so. Further, as discussed earlier, there would no motivation to include oligonucleotide probes in the PCR, since one of skill in the art at the time of Applicants' invention would have believed that doing so would merely increase the occurrence of unwanted side products.

Applicants discovered that pooling a large number of single plex quantitative PCR kits (a primer pair and a single oligonucleotide probe in each kit) into a single very large multiplex reaction could be performed, and by using for example low primer concentrations and a limited number of PCR cycles in a first highly multiplexed PCR, highly quantitative results could be obtained in a second PCR. This second PCR can be a single-plex, and can include one of the very same kits (primer pair and oligonucleotide probe) that were present in the initial pooled collection of kits. When this approach was attempted, highly accurate results were obtained. These results were truly unexpected, since the conventional wisdom held that multiplexed PCR produced unwanted side products, and adding additional nucleic acids (e.g. oligonucleotide probes) to an already complex reaction mixture would surely just make more unwanted side products. Further, oligonucleotide probes are expensive to manufacture; adding them unnecessarily to a reaction would be economically wasteful. Again, there would be no motivation for performing this combination.

Simply put, one of skill in the art of molecular biology and multiplexed PCR, at the time of Applicants' invention, would not have reasonably been motivated to take a highly

complicated reaction (a multiplexed PCR) and make it even more complicated (by adding oligonucleotide probes). Performing such a combination would be believed to simply produce a lot of unwanted non-specific side products, and would be considered a wasteful use of expensive-to-manufacture oligonucleotide probes.

One benefit of Applicants' approach is that it allows pre-existing kits (e.g.-single-plex PCR kits containing a primer pair and an oligonucleotide probe) to simply be pooled together to perform the highly multiplexed reaction, and then, those same kits in single-plex form can be used in the second PCR to accurately quantify the target nucleic acid. No additional kits that lack the oligonucleotide probes need to be manufactured. When dealing with thousands and thousands of kits, this provides an enormous manufacturing advantage. Further, the ability to accurately perform highly multiplexed quantitative PCR is unprecedented.

Concluding Remarks

Applicants here first illustrated that every element of their claimed invention was not present in the references cited by the Examiner. Alone, this is sufficient to successfully attack the Examiner's obviousness rejection. However, for the sake of expediting prosecution, Applicants went on to show that one of skill in the art would not have had a reasonable expectation that the Examiner's proposed combination would have worked. Applicants also have shown that one of skill in the art would not have had a reason to make the combination in view of the relevant teachings in the art. Thus, for at least these reasons, the Examiner has not provided a prima facie showing of obviousness. Applicants request that the rejection be withdrawn and the claims allowed.

As a final note, though the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, Applicants are not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. Applicants reserve the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that Applicants have made any disclaimers or disavowals of any subject matter supported by the present application.

PETITION FOR EXTENSION OF TIME AND FEE AUTHORIZATION

Applicants petition for a 3-month Extension of Time. The Commissioner is hereby authorized to charge the Extension of Time fee of $1,050.00 and any other fees required to Applied Biosystems Deposit Account No. 01-2213 (**Order No. 4944**)

Respectfully submitted,

Date: February 1, 2008

/Andrew K. Finn/
Andrew K. Finn, Reg. No. 54,097
Agent for Applicants

CORRESPONDENCE ADDRESS
Customer Number 22896
Applera Corporation
Applied Biosystems Division
850 Lincoln Centre Drive M/S 432-2
Foster City, California 94404
TEL: 650-554-3392
FAX: 650-638-6677